SHORT NOTE

STRUCTURAL CHANGE OF INTRACELLULAR WATER IN CAFFEINE-CONTRACTED MUSCLE CELLS

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Normal ice is hexagonal in structure and grows in a feather-like branching pattern much like that found in snowflakes.' The shape of ice formed in living cells, however, has long been known to differ in structure from normal ice and to vary accordingto the type of cell.[2] At −2°C, ice forms in frog muscle cells in the shape of long spikes when seeding ice crystals are brought into contact with the exposed cytoplasm (Fig. 1A).[2,3] Many such spikes may form at the same time; they run more or less parallel courses and do not branch or anastomose. Menz and Luyet showed that the width of the spike decreases with decreasing temperature and with increasing speed of freezing until the spike width reaches the dimension of the spacing between myofilaments (e.g., 200 Å). With further increasc in the speed of cooling, ice spikes become no longer discernible.[4,5]

The direction of the spikes, normally straight, is not an inherent property of the ice crystal. In twisted muscle fiber, the unbranching ice spikes are also twisted (Fig. 1B, see Ref. 2 also).

Living cells, as a rule, contain about 20% proteins and 80% water. Concentrated as the protein is, its distribution is discontinuous. It is water that constitutes the continuous "phase" of the cell. The major proteins of muscle cells (actin and myosin) are organized in the form of long parallel columns or filaments about 100 Å in diameter separated at regular distances of some 100 to 300 Å from their immediate neighbors in an ocean of water.[6] Thus, if the bulk of cell water is normal, the initial growth of the ice crystals within the cell should proceed in all directions. The shape of the ice formed may be distorted, but it should have geometry fundamentally similar to normal ice as seen in protein solution.[3,5] Neither prediction is borne out.

To explain the anisotropic initial growth everywhere in a resting muscle cell and to explain the lack of branching in the ice formed, it is necessary to assume that there is anisotropy in the bulk of cytoplasmic water. Since water in its normal liquid state is not anisotropic, it was suggested that the bulk of cell water might not exist in the same physical state as liquid water.[1,7-9] This suggestion is in harmony with conclusions from nuclear magnetic resonance studies."

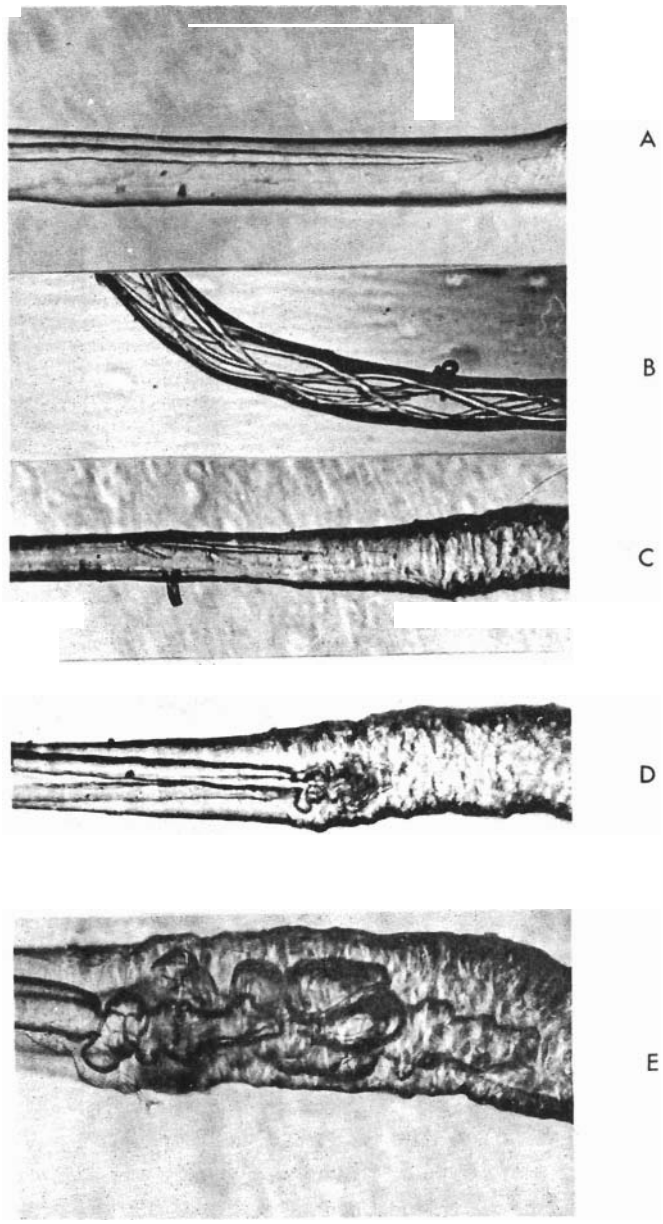

Figure 1. Patterns of ice formation in single isolated bullfrog muscle cells. All muscle fibers after isolation were blotted on wet filter paper and allowed to dry for 15 seconds in the air to minimize surface ice formation which tends to obscurevision and to prevent simultaneous formation of too many spikes.

A. Normal growth at $-2.5^{\circ}C$.
B. Growth in twisted fiber, $-2^{\circ}C$.
C. Two spikes growing toward caffeine-contracted part, $-2.5^{\circ}C$.
D. Same as C, after entering contracted region, 20 seconds after C.
E. Final state of contracted region after ice growth had slowed to negligible rate, 10 min after D.

How this physical state of cell water may be the consequence of multilayer polarizations by the protein surfaces is part of the association-induction hypothesis.[1,9,11] In brief, ice crystals run parallel to the protein filaments because they are most likely to develop at locations farther away and, thus, in between the protein filaments where the polarization is weakest. In muscle cells, the protein filaments in the form of myofibrils are oriented parallel to one another in the direction of the long axis of the cells, hence the long ice spikes. These spikes are straight or twisted depending on how the protein filaments are straight or twisted. At temperatures not too far below 0°C (e.g., −2°C), there is considerable diffusional motion of individual water molecules; such diffusion makes possible the thickening of the spike by the deposition of water moiecules on the side of ice spikes already formed. At very low temperatures when such molecular diffusion becomes greatly slowed down, narrower spikes are then observed. When this low temperature is brought about very rapidly, no ice formation was discernible. Since normal water cannot be supercooled below −40°C, this lack of ice formation at −150°C offers a strong support for the theory.

This theory also predicts that the functional activities of the living cell may involve changes of the cell proteins accompanied by changes in the physical state of the cell water.'' Figure 1C shows that an unbranching ice spike propagated in a normal manner along the untreated part of a muscle fiber until it reached theregion which had contracted in response to previous exposure to 5 mM caffeine[12] (Fig. 1D). When the spike reached this region, branching and lateral growth began; the ice formed eventually took on the irregular shape shown in Figure 1E.

In Chambers and Hale's original report and in many of our observations of normal resting muscle cells, initiation of intracellular ice formation by frozen extracellular fluid (or vice versa) was not observed.'' In caffeine-contracted muscle, on the contrary, seeding of extracellular ice formation by intracellular ice has been frequently seen. Thus, lateral growth in caffeine-treated muscle occurs in the cytoplasm as well as through the cell surface.

The data suggest that a profound change in the water structure within the cytoplasm and in the cell surface occurs during chemical excitation. It is interesting to note that previous nuclear magnetic resonance studies of nerve[13] and muscle[14] also indicate alteration of water structure in response to electrical and chemical stimulation.

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